An Intelligent Channel (and More)

Maurice Hofnung

Like a frontier, the cell membrane has two complementary functions: isolation of the cell cytoplasm from the outside by a phospholipid bilayer and control of exchanges between the cell and the environment by protein transporters. Transporters are enzymes that catalyze the transmembrane movement of their substrate. In the simplest case, the transporter behaves as a passive pore, and the driving force for transport is diffusion down a concentration gradient of substrate. More elaborate transporters move their substrates against a concentration gradient with the help of energy-coupling mechanisms (*1*).

Transporters are trans-membrane proteins, so that their x-ray structures are difficult to determine. Porins, a class of bacterial pores located in the protective outer membrane (OM) of Gram-negative bacteria such as *Escherichia coli*, are something of an exception, because they are produced in large amounts, can be purified in their active form from bacterial OMs, and are robust. To elucidate the functions of porins is not only important in understanding transport; it is also critical medically as it may reveal how to subvert these proteins with specially devised antibiotics.

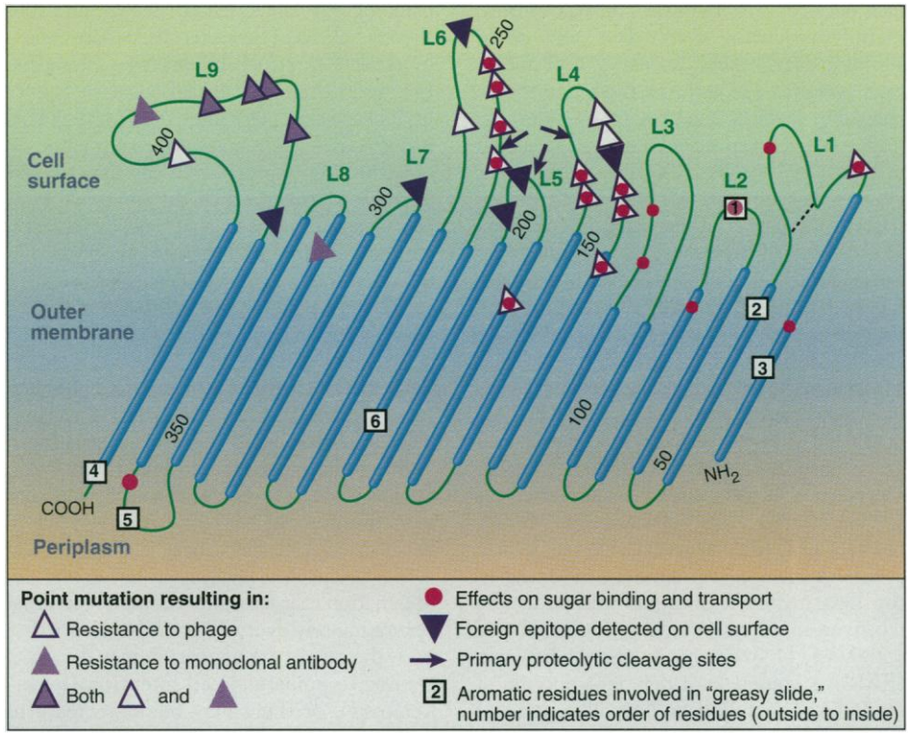
Recently, the x-ray structures of several general porins have been established (*2*, *3*). These are porins for which no substrate binding site was known and that transport hydrophilic compounds according to molecular weight (usually less than 600) or charge. Now, Schirmer and co-workers report in this issue the structure of a specific porin (*4*) which, in addition to behaving like a general porin, discriminates in a more sophisticated way, binding and specifically transporting maltose and maltodextrins (polymers of two or more glucose units). This porin—variously called lambda receptor, LamB protein, or maltoporin—has already been the object of intensive genetic and functional studies.

LamB was initially identified as an OM protein needed for infection by the bacterial virus lambda and containing the virus "receptor site." Concurrently, it was shown that the LamB protein was involved in the entry of maltose and maltodextrins into *E. coli*—hence its other name, maltoporin. The binding of substrates, a prerequisite for their specific diffusion, led to definition of the "sugar binding site" (*6*). By gene sequencing, the two sites were shown to be partially overlapping. Because the phage receptor site was thought to be exposed on the cell surface and the sugar binding site was assumed to be within the channel, it was proposed that the overlap could correspond to a "filter," located at the cell surface and participating in the access of the sugar to the binding site, possibly by orienting the sugar (*7*). The new x-ray structural model provides material support to these notions and confirms the general three-dimensional organization of porins.

Active maltoporin is a trimer; the monomer is an 18-strand β barrel, rather than the 16-stranded structure of general porins. Each monomer contains an independent channel, as predicted by biochemical (*8*) and genetic (*9*) experiments. [All three monomers of a trimer are needed for phage adsorption (*10*).] The connections between successive strands toward the cell surface are long loops, whereas the periplasmic connections are turns. The third surface loop, L3, is entirely folded into the barrel, while L1 and L6 from the same monomer and L2 from an adjacent one fold inward to different extents, forming a constriction toward the middle of the channel. The other loops form a sort of umbrella covering the entrance of most of the channel. This may be why an effect of loop L9 on transport was detected (*11*). Maltoporin has about 100 more residues than the general porins of known structure. This accounts for the two extra β strands, for the larger size of some of the loops, and possibly for the substrate specificity.

This model leads to an attractive hypothesis for specific diffusion. There is a series of six aromatic residues that compose a left-handed helical path from the inlet to the outlet of the channel. This path (the "greasy slide") guides the diffusion of the sugar by virtue of stacking interactions. Other, mostly ionizable residues in the vicinity of the path could account for the stereospecificity of the channel; binding would occur at the constriction zone. Maltopentose has a length of about 25 Å while the smallest diameter of the channel is about 5 Å. The passage of such a sugar through maltoporin is more like the movement of a spaghetti strand than of a ball. The initial orientation could be ensured by the filter.

The x-ray model provides some clues as to the validity of previous models proposed for the folding of LamB. Essentially all experimental approaches, including the newly devised insertion and detection of foreign



Schematic of the maltoporin monomer. The 421 amino acid residues are oriented from right (amino terminus) to left (carboxyl terminus). The barrel corresponding to a monomer has been opened and flattened on the page. Thick lines, residues in transmembrane β strands; thin lines, loops and turns. The triangles and arrows designate regions assumed to be near or at the cell surface [data reproduced from (*7*, *12*) and references therein]. Original courtesy of T. Schirmer.

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antigenic determinants (*12*), have led to correct conclusions about the topology of LamB (see figure). Most of the residues detected as part of the maltodextrin binding site are located within the channel, although often in the inward parts of loops. Secondary structure predictions, by themselves, were poor in establishing correct topology; recent methods based on the three-dimensional structures of other porins were more successful (*8*) but still predicted 16 transmembranous strands instead of 18.

This model raises interesting questions. How does the helical path work? How do other sugars that appear to rely on LamB for growth in limiting conditions (*13*) diffuse? How would a filter work? Is it an early weak binding site? The notion that the general porins may generate a local electric field near the channel constriction, sufficient to orient small hydrophilic molecules and repel hydrophobic ones (*2*), suggests that there is only a quantitative difference between binding sites and filters. What is the role of the umbrella? Does it protect the channel and binding site from noxious agents or from phages (the loops of the umbrella are the parts of LamB that are the most variable between bacterial species)? Do the loops move in vivo and thus contribute to the motion of the sugar? Is there a structural connection between LamB and other components of the maltose transport system? What exactly is the phage receptor—a binding site for the phage or an accessibility gate to the real, possibly yet undetected, binding site (*14*)? How does this fast, tight interaction succeed in firmly attaching two large structures such as a bacteria and a phage? Is there a relation between the pathway followed by the phage DNA upon infection and LamB organization? As usual, a nice achievement in science provides at least as many questions as answers.

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Defects in the Barrier

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The skin is a physical barrier at the interface between an organism and its environment—preventing water loss and withstanding mechanical, chemical, and microbial assaults. To perform these functions, the outer layer of the skin (the epidermis) undergoes keratinization, a process in which epidermal cells progressively mature from basal cells with proliferative potential to lifeless flattened squames of the stratum corneum. During this differentiation, certain genes are activated while others are down-regulated—leading to changes in both structural proteins and in the expression and activation of enzymes that control posttranslational modifications, metabolic changes, and lipid synthesis. A defect in any one of these structural components or enzymatic processes has the potential to impair the barrier function of the skin and cause disease.

Surprisingly, the phenotypic consequences of defects in many of these diverse processes can be very similar—resulting in the keratinization disorders known as the ichthyoses, characterized by thickened and scaly skin (*1*): Recessive X-linked ichthyosis (RXLI) is caused by a deficient enzyme of cholesterol metabolism (steroid sulfatase) (*2*); the epidermolytic ichthyoses—epidermolytic hyperkeratosis (EHK), epidermolytic palmoplantar keratoderma (EPPK), and ichthyosis bullosa of Siemens (IBS)—are caused by defects in structural proteins (keratins) (*3*); and as reported in this issue of *Science* by Huber *et al.* (*4*), lamellar ichthyosis (LI) is due to defects in an enzyme that catalyzes cross-linking of proteins in the upper layers of the epidermis (a transglutaminase). How can defects in genes that encode proteins with such different functions produce skin disorders that are clinically so similar?

The epidermis is a perpetually renewing tissue, comprised of four histologically distinct cellular layers, each with a distinct maturation state of the keratinocyte, the major cell type of the epidermis (see figure). Keratinocytes arise from stem cells in the basal layer, and move through a series of differentiation events until they are finally sloughed into the environment (desquamation). Thus in the normal epidermis, there is a balance between the processes of proliferation and desquamation that results in a complete renewal approximately every 28 days. In the ichthyoses, the rate of desquamation may decrease, leading to epidermal cell retention (hyperkeratosis), or there may be an increase in proliferation, which further exacerbates the build up of skin cells in these patients (or both processes may occur simultaneously.)

Keratins are major structural proteins synthesized in keratinocytes. They assemble into a weblike pattern of intermediate filaments (IFs) that emanate from a perinuclear ring, extend throughout the cytoplasm, and terminate at junctional complexes called desmosomes and hemidesmosomes (*5*). Keratin IFs are essential for maintaining the integrity of the epidermis; mutations in six keratin genes result in four distinct epidermal diseases (*3*). All of these disorders are characterized by blistering, with lesions originating at the site of synthesis of the mutant keratin: Epidermolysis bullosa simplex (EBS) shows mutations in the basal layer keratins K5 or K14; EHK has spinous layer K1 or K10 defects; IBS has granular layer K2e defects; and EPPK has granular layer K9 defects that are restricted to palmar and plantar epidermis. Interestingly, only those diseases with defects in upper layer keratins (EHK, IBS, EPPK) exhibit hyperkeratosis, or a thickening of the stratum corneum. Why keratinization disorders only result from defects in keratin genes expressed in the differentiated layers of the epidermis is not clear, but there are some clues. Lysis of differentiated keratinocytes may release cytokines involved in the wound response such as transforming growth factor α (TGF-α). Given that TGF-α and its receptor are upregulated in EHK lesions (*6*) and that overexpression of TGF-α in the epidermis of transgenic mice produces a marked hyperkeratosis (*7*), it is likely that TGF-α contributes to this aspect of the disease. In addition, during the final stages of normal differentiation, keratin IFs are aligned into highly ordered and condensed arrays through interactions with filaggrin, a matrix protein (*8*). In the keratin disorders, the IF networks collapse around the nucleus, preventing at-

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